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TITLE: A.beta. peptides that modulate .beta.-amyloid aggregation

US PATENT NO. (1):
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Brief Summary Text (4):
Pathologically, AD is characterized by the presence of distinctive lesions in the victim's brain. These brain lesions include abnormal intracellular filaments called neurofibrillary tangles (NTFs) and extracellular deposits of amyloidogenic proteins in senile, or amyloid, plaques. Amyloid deposits are also present in the walls of cerebral blood vessels of AD patients. The major protein constituent of amyloid plaques has been identified as a 4 kilodalton peptide called .beta.-amyloid peptide (.beta.-AP)(Glenner, G. G. and Wong, C. W. (1984) Biochem. Biophys. Res. Commun. 120:885-890; Masters, C. et al. (1985) Proc. Natl. Acad. Sci. USA 82:4245-4249). Diffuse deposits of .beta.-AP are frequently observed in normal adult brains, whereas AD brain tissue is characterized by more compacted, dense-core .beta.-amyloid plaques. (See e.g., Davies, L. et al. (1988) Neurology 38:1688-1693). These observations suggest that .beta.-AP deposition precedes, and contributes to, the destruction of neurons that occurs in AD. In further support of a direct pathogenic role for .beta.-AP, .beta.-amyloid has been shown to be toxic to mature neurons, both in culture and in vivo. Yankner, B. A. et al. (1989) Science 245:417-420; Yankner, B. A. et al. (1990) Proc. Natl. Acad. Sci. USA 87:9020-9023; Roher, A. E. et al. (1991) Biochem. Biophys. Res. Commun. 174:572-579; Kowall, N. W. et al. (1991) Proc. Natl. Acad. Sci. USA 88:7247-7251. Furthermore, patients with hereditary cerebral hemorrhage with amyloidosis-Dutch-type (HCHWA-D), which is characterized by diffuse .beta.-amyloid deposits within the cerebral cortex and cerebrovasculature, have been shown to have a point mutation that leads to an amino acid substitution within .beta.-AP. Levy, E. et al. (1990) Science 248:1124-1126. This observation demonstrates that a specific alteration of the .beta.-AP sequence can cause .beta.-amyloid to be deposited.

Brief Summary Text (5):
Natural .beta.-AP is derived by proteolysis from a much larger protein called the amyloid precursor protein (APP). Kang, J. et al. (1987) Nature 325:733; Goldgaber, D. et al. (1987) Science 235:877; Robakis, N. K. et al. (1987) Proc. Natl. Acad. Sci. USA 84:4190; Tanzi, R. E. et al. (1987) Science 235:880. The APP gene maps to chromosome 21, thereby providing an explanation for the .beta.-amyloid deposition seen at an early age in individuals with Down's syndrome, which is caused by trisomy of chromosome 21. Mann, D. M. et al. (1989) Neuropathol. Appl. Neurobiol. 15:317; Rumble, B. et al. (1989) N. Eng. J Med. 320:1446. APP contains a single membrane spanning domain, with a long amino terminal region (about two-thirds of the protein) extending into the extracellular environment and a shorter carboxy-terminal region projecting into the cytoplasm. Differential splicing of the APP messenger RNA leads to at least five forms of APP, composed of either 563 amino acids (APP-563), 695 amino acids (APP-695), 714 amino acids (APP-714), 751 amino acids (APP-751) or 770 amino acids (APP-770).

Brief Summary Text (6):
Within APP, naturally-occurring .beta. amyloid peptide begins at an aspartic acid residue at amino acid position 672 of APP-770. Naturally-occurring .beta.-AP derived from proteolysis of APP is 39 to 43 amino acid residues in length, depending on the carboxy-terminal end point, which exhibits heterogeneity. The predominant circulating form of .beta.-AP in the blood and cerebrospinal fluid of both AD patients and normal adults is .beta.1-40 ("short .beta."). Seubert, P. et al. (1992) Nature 359:325; Shoji, M. et al. (1992) Science 258:126. However, .beta.1-42 and .beta.1-43 ("long .beta.") also are forms in .beta.-amyloid plaques. Masters, C. et al. (1985) Proc. Natl Acad.

Sci. USA 82:4245; Miller, D. et al. (1993) Arch. Biochem. Biophys. 301:41; Mori, H. et al. (1992) J. Biol. Chem. 267:17082. Although the precise molecular mechanism leading to .beta.-APP aggregation and deposition is unknown, the process has been likened to that of nucleation-dependent polymerizations, such as protein crystallization, microtubule formation and actin polymerization. See e.g., Jarrett, J. T. and Lansbury, P. T. (1993) Cell 73:1055-1058. In such processes, polymerization of monomer components does not occur until nucleus formation. Thus, these processes are characterized by a lag time before aggregation occurs, followed by rapid polymerization after nucleation. Nucleation can be accelerated by the addition of a "seed" or preformed nucleus, which results in rapid polymerization. The long .beta. forms of .beta.-AP have been shown to act as seeds, thereby accelerating polymerization of both long and short .beta.-AP forms. Jarrett, J. T. et al. (1993) Biochemistry 32:4693.

Brief Summary Text (7):
In one study, in which amino acid substitutions were made in .beta.-AP, two mutant .beta. peptides were reported to interfere with polymerization of non-mutated .beta.-AP when the mutant and non-mutant forms of peptide were mixed. Hilbich, C. et al. (1992) J. Mol. Biol. 228:460-473. However, equimolar amounts of the mutant and non-mutant (i.e., natural) .beta. amyloid peptides were used to see this effect and the mutant peptides were reported to be unsuitable for use in vivo. Hilbich, C. et al. (1992), supra.

Brief Summary Text (10):
In the most preferred embodiment of the invention, the compound modulates the aggregation of natural .beta.-AP. The invention provides a .beta.-amyloid peptide compound comprising a formula: ##STR1## wherein Xaa is a .beta.-amyloid peptide having an amino-terminal amino acid residue corresponding to position 668 of .beta.-amyloid precursor protein-770 (APP-770) or to a residue carboxy-terminal to position 668 of APP-770, A is a modifying group attached directly or indirectly to the .beta.-amyloid peptide of the compound such that the compound inhibits aggregation of natural .beta.-amyloid peptides when contacted with the natural .beta.-amyloid peptides, and n is an integer selected such that the compound inhibits aggregation of natural .beta.-amyloid peptides when contacted with the natural .beta.-amyloid peptides.

Brief Summary Text (11):
In one embodiment, at least one A group is attached directly or indirectly to the amino terminus of the .beta.-amyloid peptide of the compound. In another embodiment, at least one A group is attached directly or indirectly to the carboxy terminus of the .beta.-amyloid peptide of the compound. In yet another embodiment, at least one A group is attached directly or indirectly to a side chain of at least one amino acid residue of the .beta.-amyloid peptide of the compound.

Brief Summary Text (12):
The invention also provides a .beta.-amyloid modulator compound comprising an A.beta. aggregation core domain (ACD) coupled directly or indirectly to at least one modifying group (MG) such that the compound modulates the aggregation or inhibits the neurotoxicity of natural .beta.-amyloid peptides when contacted with the natural .beta.-amyloid peptides. Preferably, the A.beta. aggregation core domain is modeled after a subregion of natural .beta.-amyloid peptide between 3 and 10 amino acids in length.

Brief Summary Text (13):
The invention also provides .beta.-amyloid modulator compound comprising a formula: ##STR2## wherein Xaa.sub.1, Xaa.sub.2 and Xaa.sub.3 are each amino acid structures and at least two of Xaa.sub.1, Xaa.sub.2 and Xaa.sub.3 are, independently, selected from the group consisting of a leucine structure, a phenylalanine structure and a valine structure;

Brief Summary Text (14):
Y, which may or may not be present, is a peptidic structure having the formula (Xaa).sub.a, wherein Xaa is any amino acid structure and a is an integer from 1 to 15;

Brief Summary Text (15):
Z, which may or may not be present, is a peptidic structure having the formula (Xaa).sub.b, wherein Xaa is any amino acid structure and b is an integer from 1 to 15; and

Brief Summary Text (18):
The invention further provides a .beta.-amyloid modulator compound comprising a

formula: ##STR3## wherein Xaa.sub.1 and Xaa.sub.3 are amino acid structures; Xaa.sub.2 is a valine structure;

Brief Summary Text (20):
Y, which may or may not be present, is a peptidic structure having the formula (Xaa).sub.a, wherein Xaa is any amino acid structure and a is an integer from 1 to 15;

Brief Summary Text (21):
Z, which may or may not be present, is a peptidic structure having the formula (Xaa).sub.b, wherein Xaa is any amino acid structure and b is an integer from 1 to 15; and

Brief Summary Text (37):
The invention still further provides a .beta.-amyloid modulator compound comprising a modifying group attached directly or indirectly to a peptidic structure, wherein the peptidic structure comprises amino acid structures having an amino acid sequence selected from the group consisting of His-Gln-Lys-Leu-Val-Phe-Phe-Ala (SEQ ID NO:5), His-Gln-Lys-Leu-Val-Phe-Phe (SEQ ID NO:6), Gln-Lys-Leu-Val-Phe-Phe-Ala (SEQ ID NO:7), Gln-Lys-Leu-Val-Phe-Phe (SEQ ID NO:8), Lys-Leu-Val-Phe-Phe-Ala (SEQ ID NO:9), Lys-Leu-Val-Phe-Phe (SEQ ID NO:10), Leu-Val-Phe-Phe-Ala (SEQ ID NO:11), Leu-Val-Phe-Phe (SEQ ID NO:12), Leu-Ala-Phe-Phe-Ala (SEQ ID NO:13), Val-Phe-Phe (SEQ ID NO:19), Phe-Phe-Ala (SEQ ID NO:20), Phe-Phe-Val-Leu-Ala (SEQ ID NO:21), Leu-Val-Phe-Phe-Lys (SEQ ID NO:22), Leu-Val-Iodotyrosine-Phe-Ala (SEQ ID NO:23), Val-Phe-Phe-Ala (SEQ ID NO:24), Ala-Val-Phe-Phe-Ala (SEQ ID NO:25), Leu-Val-Phe-Iodotyrosine-Ala (SEQ ID NO:26), Leu-Val-Phe-Phe-Ala-Glu (SEQ ID NO:27), Phe-Phe-Val-Leu (SEQ ID NO:28), Phe-Lys-Phe-Val-Leu (SEQ ID NO:29), Lys-Leu-Val-Ala-Phe (SEQ ID NO:30), Lys-Leu-Val-Phe-Phe-.beta.Ala (SEQ ID NO:31) and Leu-Val-Phe-Phe-DAla (SEQ ID NO:32).

Brief Summary Text (41):
The invention also provides a .beta.-amyloid peptide compound comprising an amino acid sequence having at least one amino acid deletion compared to .beta.AP.sub.1-39, such that the compound inhibits aggregation of natural .beta.-amyloid peptides when contacted with the natural .beta.-amyloid peptides. In one embodiment, the compound has at least one internal amino acid deleted compared to .beta.AP.sub.1-39. In another embodiment, the compound has at least one N-terminal amino acid deleted compared to .beta.AP.sub.1-39. In yet another embodiment, the compound has at least one C-terminal amino acid deleted compared to .beta.AP.sub.1-39. Preferred compounds include .beta.AP.sub.6-20 (SEQ ID NO:4), .beta.AP.sub.16-30 (SEQ ID NO:14), .beta.AP.sub.1-20, 26-40 (SEQ ID NO:15) and EEVVHHHHQQ-.beta.AP.sub.16-40 (SEQ ID NO:16).

Detailed Description Text (2):
This invention pertains to compounds, and pharmaceutical compositions thereof, that can modulate the aggregation of amyloidogenic proteins and peptides, in particular compounds that can modulate the aggregation of natural D amyloid peptides (.beta.-AP) and inhibit the neurotoxicity of natural .beta.-APs. A compound of the invention that modulates aggregation of natural .beta.-AP, referred to herein interchangeably as a .beta. amyloid modulator compound, a .beta. amyloid modulator or simply a modulator, alters the aggregation of natural .beta.-AP when the modulator is contacted with natural .beta.-AP. Thus, a compound of the invention acts to alter the natural aggregation process or rate for .beta.-AP, thereby disrupting this process. Preferably, the compounds inhibit .beta.-AP aggregation. Furthermore, the invention provides subregions of the .beta. amyloid peptide that are sufficient, when appropriately modified as described herein, to alter (and preferably inhibit) aggregation of natural .beta. amyloid peptides when contacted with the natural .beta. amyloid peptides. In particular, preferred modulator compounds of the invention are comprised of a modified form of an A.beta. aggregation core domain, modeled after the aforementioned A.beta. subregion (as described further below), which is sufficient to alter (and preferably inhibit) the natural aggregation process or rate for .beta.-AP. This A.beta. aggregation core domain can comprises as few as three amino acid residues (or derivative, analogues or mimetics thereof). Moreover, while the amino acid sequence of the A.beta. aggregation core domain can directly correspond to an amino acid sequence found in natural .beta.-AP, it is not essential that the amino acid sequence directly correspond to a .beta.-AP sequence. Rather, amino acid residues derived from a preferred subregion of .beta.-AP (a hydrophobic region centered around positions 17-20) can be rearranged in order and/or substituted with homologous residues within a modulator compound of the invention and yet maintain their inhibitory activity (described further below).

Detailed Description Text (5):

The terms "natural .beta.-amyloid peptide", "natural .beta.-AP" and "natural A.beta. peptide", used interchangeably herein, are intended to encompass naturally occurring proteolytic cleavage products of the .beta. amyloid precursor protein (APP) which are involved in .beta.-AP aggregation and .beta.-amyloidosis. These natural peptides include .beta.-amyloid peptides having 39-43 amino acids (i.e., A.beta..sub.1-39, A.beta..sub.1-40, A.beta..sub.1-41, A.beta..sub.1-42 and A.beta..sub.1-43). The amino-terminal amino acid residue of natural .beta.-AP corresponds to the aspartic acid residue at position 672 of the 770 amino acid residue form of the amyloid precursor protein ("APP-770"). The 43 amino acid long form of natural .beta.-AP has the amino acid sequence.

Detailed Description Text (7):
(also shown in SEQ ID NO:1), whereas the shorter forms have 1-4 amino acid residues deleted from the carboxy-terminal end. The amino acid sequence of APP-770 from position 672 (i.e., the amino-terminus of natural .beta.-AP) to its C-terminal end (103 amino acids) is shown in SEQ ID NO:2. The preferred form of natural .beta.-AP for use in the aggregation assays described herein is A.beta..sub.1-40.

Detailed Description Text (15):
Preferably, .beta.-amyloid peptide of the compound has an amino-terminal amino acid residue corresponding to position 668 of .beta.-amyloid precursor protein-770 (APP-770) or to a residue carboxy-terminal to position 668 of APP-770. The amino acid sequence of APP-770 from position 668 to position 770 (i.e., the carboxy terminus) is shown below and in SEQ ID NO:2:

Detailed Description Text (17):
More preferably, the amino-terminal amino acid residue of the .beta.-amyloid peptide corresponds to position 672 of APP-770 (position 5 of the amino acid sequence of SEQ ID NO:2) or to a residue carboxy-terminal to position 672 of APP-770. Although the .beta.-amyloid peptide of the compound may encompass the 103 amino acid residues corresponding to positions 668-770 of APP-770, preferably the peptide is between 6 and 60 amino acids in length, more preferably between 10 and 43 amino acids in length and even more preferably between 10 and 25 amino acid residues in length.

Detailed Description Text (18):
As used herein, the term ".beta. amyloid peptide", as used in a modulator of the invention is intended to encompass peptides having an amino acid sequence identical to that of the natural sequence in APP, as well as peptides having acceptable amino acid substitutions from the natural sequence. Acceptable amino acid substitutions are those that do not affect the ability of the peptide to alter natural .beta.-AP aggregation. Moreover, particular amino acid substitutions may further contribute to the ability of the peptide to alter natural .beta.-AP aggregation and/or may confer additional beneficial properties on the peptide (e.g., increased solubility, reduced association with other amyloid proteins, etc.). For example, substitution of hydrophobic amino acid residues for the two phenylalanine residues at positions 19 and 20 of natural .beta.-AP (positions 19 and 20 of the amino acid sequence shown in SEQ ID NO:1) may further contribute to the ability of the peptide to alter .beta.-AP aggregation (see Hilbich, C. (1992) J. Mol. Biol. 228:460-473). Thus, in one embodiment, the .beta.-AP of the compound consists of the amino acid sequence shown below and in SEQ ID NO:3:

Detailed Description Text (20):
(or an amino-terminal or carboxy-terminal deletion thereof), wherein Xaa is a hydrophobic amino acid. Examples of hydrophobic amino acids are isoleucine, leucine, threonine, serine, alanine, valine or glycine. Preferably, F.sub.19 F.sub.20 is substituted with T.sub.19 T.sub.20 or G.sub.19 I.sub.20.

Detailed Description Text (21):
Other suitable amino acid substitutions include replacement of amino acids in the human peptide with the corresponding amino acids of the rodent .beta.-AP peptide. The three amino acid residues that differ between human and rat .beta.-AP are at positions 5, 10 and 13 of the amino acid sequence shown in SEQ ID NOs:1 and 3. A human .beta.-AP having the human to rodent substitutions Arg.sub.5 to Gly, Tyr.sub.10 to Phe and His.sub.13 to Arg has been shown to retain the properties of the human peptide (see Fraser, P. E. et al. (1992) Biochemistry 31:10716-10723; and Hilbich, C. et al. (1991) Eur. J. Biochem. 201:61-69). Accordingly, a human .beta.-AP having rodent .beta.-AP a.a. substitutions is suitable for use in a modulator of the invention.

Detailed Description Text (22):
Other possible .beta.-AP amino acid substitutions are described in Hilbich, C. et al.

(1991) J. Mol. Biol. 218:149-163; and Hilbich, C. (1992) J. Mol. Biol. 228:460-473. Moreover, amino acid substitutions that affect the ability of .beta.-AP to associate with other proteins can be introduced. For example, one or more amino acid substitutions that reduce the ability of .beta.-AP to associate with the serpin enzyme complex (SEC) receptor, .alpha.1-antichymotrypsin (ACT) and/or apolipoprotein E (ApoE) can be introduced. A preferred substitution for reducing binding to the SEC receptor is L.sub.34 M.sub.35 to A.sub.34 A.sub.35 (at positions 34 and 35 of the amino acid sequences shown in SEQ ID NOs:1 and 3). A preferred substitution for reducing binding to ACT is S.sub.8 to A.sub.8 (at position 8 of the amino acid sequences shown in SEQ ID NOs:1 and 3).

Detailed Description Text (23):
Alternative to .beta.-AP amino acid substitutions described herein or known in the art, a modulator composed, at least in part, of an amino acid-substituted .beta. amyloid peptide can be prepared by standard techniques and tested for the ability to alter .beta.-AP aggregation using an aggregation assay described herein. To retain the properties of the original modulator, preferably conservative amino acid substitutions are made at one or more amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art, including basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g. glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), .beta.-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Accordingly, a modulator composed of a .beta. amyloid peptide having an amino acid sequence that is mutated from that of the wild-type sequence in APP-770 yet which still retains the ability to alter natural .beta.-AP aggregation is within the scope of the invention.

Detailed Description Text (27):
In yet another embodiment, the modulating group is attached to the side chain of at least one amino acid residue of the .beta.-amyloid peptide of the compound (e.g., through the epsilon amino group of a lysyl residue(s), through the carboxyl group of an aspartic acid residue(s) or a glutamic acid residue(s), through a hydroxy group of a tyrosyl residue(s), a serine residue(s) or a threonine residue(s) or other suitable reactive group on an amino acid side chain).

Detailed Description Text (32):
Additional suitable modulating groups may include other cyclic and heterocyclic compounds and other compounds having similar steric "bulk". Non-limiting examples of compounds which can be used to modify a .beta.-AP are shown schematically in FIG. 2, and include N-acetylneuraminic acid, cholic acid, trans-4-cotininecarboxylic acid, 2-imino-1-imidazolidineacetic acid, (S)-(-)-indoline-2-carboxylic acid, (-)-menthoxyacetic acid, 2-norbornaneacetic acid, .gamma.-oxo-5-acenaphthenebutyric acid, (-)-2-oxo-4-thiazolidinecarboxylic acid, tetrahydro-3-furoic acid, 2-iminobiotin-N-hydroxysuccinimide ester, diethylenetriaminepentaacetic dianhydride, 4-morpholinecarbonyl chloride, 2-thiopheneacetyl chloride, 2-thiophenesulfonyl chloride, 5-(and 6-)-carboxyfluorescein (succinimidyl ester), fluorescein isothiocyanate, and acetic acid (or derivatives thereof). Suitable modulating groups are described further in subsection II below.

Detailed Description Text (35):
Although not intending to be limited by mechanism, the ACD of the modulators of the invention is thought to confer a specific targeting function on the compound that allows the compound to recognize and specifically interact with natural .beta.-AP. Preferably, the ACD is modeled after a subregion of natural .beta.-AP that is less than 15 amino acids in length and more preferably is between 3-10 amino acids in length. In various embodiments, the ACD is modeled after a subregion of .beta.-AP that is 10, 9, 8, 7, 6, 5, 4 or 3 amino acids in length. In one embodiment, the subregion of .beta.-AP upon which the ACD is modeled is an internal or carboxy-terminal region of .beta.-AP (i.e., downstream of the amino-terminus at amino acid position 1). In another embodiment, the ACD is modeled after a subregion of .beta.-AP that is hydrophobic. In certain specific embodiments, the term A.beta. aggregation core domain specifically excludes .beta.-AP subregions corresponding to amino acid positions 1-15 (A.beta..sub.1-15), 6-20 (A.beta..sub.6-20) and 16-40 (A.beta..sub.16-40).

Detailed Description Text (36):

An A.beta. aggregation core domain can be comprised of amino acid residues linked by peptide bonds. That is, the ACD can be a peptide corresponding to a subregion of .beta.-AP. Alternatively, an A.beta. aggregation core domain can be modeled after the natural A.beta. peptide region but may be comprised of a peptide analogue, peptide derivative or peptidomimetic compound, or other similar compounds which mimics the structure and function of the natural peptide. Accordingly, as used herein, an "A.beta. aggregation core domain" is intended to include peptides, peptide analogues, peptide derivatives and peptidomimetic compounds which, when appropriately modified, retain the aggregation modulatory activity of the modified natural A.beta. peptide subregion. Such structures that are designed based upon the amino acid sequence are referred to herein as "A.beta. derived peptidic structures." Approaches to designing peptide analogues, derivatives and mimetics are known in the art. For example, see Farmer, P. S. in Drug Design (E. J. Ariens, ed.) Academic Press, New York, 1980, vol. 10, pp. 119-143; Ball. J. B. and Alewood, P. F. (1990) J. Mol. Recognition 3:55; Morgan, B. A. and Gainor, J. A. (1989) Ann. Rep. Med Chem. 24:243; and Freidinger, R. M. (1989) Trends Pharmacol. Sci. 10:270. See also Sawyer, T. K. (1995) "Peptidomimetic Design and Chemical Approaches to Peptide Metabolism" in Taylor, M. D. and Amidon, G. L. (eds.) Peptide-Based Drug Design: Controlling Transport and Metabolism, Chapter 17; Smith, A. B. 3rd, et al. (1995) J. Am. Chem. Soc. 117:11113-11123; Smith, A. B. 3rd, et al. (1994) J. Am. Chem. Soc. 116:9947-9962; and Hirschman, R., et al. (1993) J. Am. Chem. Soc. 115:12550-12568.

Detailed Description Text (37):
As used herein, a "derivative" of a compound X (e.g., a peptide or amino acid) refers to a form of X in which one or more reaction groups on the compound have been derivatized with a substituent group. Examples of peptide derivatives include peptides in which an amino acid side chain, the peptide backbone, or the amino- or carboxy-terminus has been derivatized (e.g., peptidic compounds with methylated amide linkages). As used herein an "analogue" of a compound X refers to a compound which retains chemical structures of X necessary for functional activity of X yet which also contains certain chemical structures which differ from X. An examples of an analogue of a naturally-occurring peptide is a peptides which includes one or more non-naturally-occurring amino acids. As used herein, a "mimetic" of a compound X refers to a compound in which chemical structures of X necessary for functional activity of X have been replaced with other chemical structures which mimic the conformation of X. Examples of peptidomimetics include peptidic compounds in which the peptide backbone is substituted with one or more benzodiazepine molecules (see e.g., James, G. L. et al. (1993) Science 260:1937-1942), peptides in which all L-amino acids are substituted with the corresponding D-amino acids and "retro-inverso" peptides (see U.S. Pat. No. 4,522,752 by Sisto), described further below.

Detailed Description Text (39):
Other possible modifications include an N-alkyl (or aryl) substitution (.psi.[CONR]), backbone crosslinking to construct lactams and other cyclic structures, substitution of all D-amino acids for all L-amino acids within the compound ("inverso" compounds) or retro-inverso amino acid incorporation (.psi.[NHCO]). By "inverso" is meant replacing L-amino acids of a sequence with D-amino acids, and by "retro-inverso" or "enantio-retro" is meant reversing the sequence of the amino acids ("retro") and replacing the L-amino acids with D-amino acids. For example, if the parent peptide is Thr-Ala-Tyr, the retro modified form is Tyr-Ala-Thr, the inverso form is thr-ala-tyr, and the retro-inverso form is tyr-ala-thr (lower case letters refer to D-amino acids). Compared to the parent peptide, a retro-inverso peptide has a reversed backbone while retaining substantially the original spatial conformation of the side chains, resulting in a retro-inverso isomer with a topology that closely resembles the parent peptide. See Goodman et al. "Perspectives in Peptide Chemistry" pp. 283-294 (1981). See also U.S. Pat. No. 4,522,752 by Sisto for further description of "retro-inverso" peptides.

Detailed Description Text (41):
In a preferred embodiment, the ACD of the modulator is modeled after the subregion of .beta.-AP encompassing amino acid positions 17-20 (i.e., Leu-Val-Phe-Phe; SEQ ID NO:12). As described further in Examples 7, 8 and 9, peptide subregions of A.beta..sub.1-40 were prepared, amino-terminally modified and evaluated for their ability to modulate aggregation of natural .beta.-amyloid peptides. One subregion that was effective at inhibiting aggregation was A.beta..sub.6-20 (i.e., amino acid residues 6-20 of the natural A.beta..sub.1-40 peptide, the amino acid sequence of which is shown in SEQ ID NO:4). Amino acid residues were serially deleted from the amino-terminus or carboxy terminus of this subregion to further delineate a minimal subregion that was sufficient for aggregation inhibitory activity. This process defined A.beta..sub.17-20 (i.e., amino acid residues 17-20 of the natural A.beta..sub.1-40 peptide) as a minimal

subregion that, when appropriately modified, is sufficient for aggregation inhibitory activity. Accordingly, an "A.beta. aggregation core domain" within a modulator compound of the invention can be modeled after A.beta..sub.17-20. In one embodiment, the A.beta. aggregation core domain comprises A.beta..sub.17-20 itself (i.e., a peptide comprising the amino acid sequence leucine-valine-phenylalanine-phenylalanine; SEQ ID NO:12). In other embodiments, the structure of A.beta..sub.17-20 is used as a model to design an A.beta. aggregation core domain having similar structure and function to A.beta..sub.17-20. For example, peptidomimetics, derivatives or analogues of A.beta..sub.17-20 (as described above) can be used as an A.beta. aggregation core domain. In addition to A.beta..sub.17-20, the natural A.beta. peptide is likely to contain other minimal subregions that are sufficient for aggregation inhibitory activity. Such additional minimal subregions can be identified by the processes described in Examples 7, 8 and 9, wherein a 15 mer subregion of A.beta..sub.1-40 is serially deleted from the amino-terminus or carboxy terminus, the deleted peptides are appropriately modified and then evaluated for aggregation inhibitory activity.

Detailed Description Text (42):
One form of the .beta.-amyloid modulator compound comprising an A.beta. aggregation core domain modeled after A.beta..sub.17-20 coupled directly or indirectly to at least one modifying group has the formula: ##STR8## wherein Xaa.sub.1 and Xaa.sub.3 are amino acid structures;

Detailed Description Text (45):
Y, which may or may not be present, is a peptidic structure having the formula (Xaa).sub.a, wherein Xaa is any amino acid structure and a is an integer from 1 to 15;

Detailed Description Text (46):
Z, which may or may not be present, is a peptidic structure having the formula (Xaa).sub.b, wherein Xaa is any amino acid structure and b is an integer from 1 to 15; and

Detailed Description Text (50):
As demonstrated in Example 9, amino acid positions 18 (Val.sub.18) and 20 (Phe.sub.20) of A.beta..sub.17-20 (corresponding to Xaa.sub.2 and Xaa.sub.4) are particularly important within the core domain for inhibitory activity of the modulator compound. Accordingly, these positions are conserved within the core domain in the formula shown above. The terms "valine structure" and "phenylalanine structure" as used in the above formula are intended to include the natural amino acids, as well as non-naturally-occurring analogues, derivatives and mimetics of valine and phenylalanine, respectively, (including D-amino acids) which maintain the functional activity of the compound. Moreover, although Val.sub.18 and Phe.sub.20 have an important functional role, it is possible that Xaa.sub.2 and/or Xaa.sub.4 can be substituted with other naturally-occurring amino acids that are structurally related to valine or phenylalanine, respectively, while still maintaining the activity of the compound. Thus, the terms "valine structure" is intended to include conservative amino acid substitutions that retain the activity of valine at Xaa.sub.2, and the term "phenylalanine structure" is intended to include conservative amino acid substitutions that retain the activity of phenylalanine at Xaa.sub.4. However, the term "valine structure" is not intended to include threonine.

Detailed Description Text (51):
In contrast to positions 18 and 20 of A.beta..sub.17-20, a Phe to Ala substitution at position 19 (corresponding to Xaa.sub.3) did not abolish the activity of the modulator, indicating position 19 may be more amenable to amino acid substitution. In various embodiments of the above formula, positions Xaa.sub.1 and Xaa.sub.3 are any amino acid structure. The term "amino acid structure" is intended to include natural and non-natural amino acids as well as analogues, derivatives and mimetics thereof, including D-amino acids. In a preferred embodiment of the above formula, Xaa.sub.1 is a leucine structure and Xaa.sub.3 is a phenylalanine structure (i.e., modeled after Leu.sub.17 and Phe.sub.19, respectively, in the natural A.beta. peptide sequence). The term "leucine structure" is used in the same manner as valine structure and phenylalanine structure described above. Alternatively, an another embodiment, Xaa.sub.3 is an alanine structure.

Detailed Description Text (52):
The four amino acid structure ACD of the modulator of the above formula can be flanked at the amino-terminal side, carboxy-terminal side, or both, by peptidic structures derived either from the natural A.beta. peptide sequence or from non-A.beta. sequences. The term "peptidic structure" is intended to include peptide analogues, derivatives and

mimetics thereof, as described above. The peptidic structure is composed of one or more linked amino acid structures, the type and number of which in the above formula are variable. For example, in one embodiment, no additional amino acid structures flank the Xaa.sub.1 -Xaa.sub.2 -Xaa.sub.3 -Xaa.sub.4 core sequence (i.e., Y and Z are absent in the above formula). In another embodiment, one or more additional amino acid structures flank only the amino-terminus of the core sequences (i.e., Y is present but Z is absent in the above formula). In yet another embodiment, one or more additional amino acid structures flank only the carboxy-terminus of the core sequences (i.e., Z is present but Y is absent in the above formula). The length of flanking Z or Y sequences also is variable. For example, in one embodiment, a and b are integers from 1 to 15. More preferably, a and b are integers between 1 and 10. Even more preferably, a and b are integers between 1 and 5. Most preferably, a and b are integers between 1 and 3.

Detailed Description Text (55):
Xaa.sub.1 and Xaa.sub.3 are amino acids or amino acid mimetics;

Detailed Description Text (58):
Y, which may or may not be present, is a peptide or peptidomimetic having the formula (Xaa).sub.a, wherein Xaa is any amino acid or amino acid mimetic and a is an integer from 1 to 15;

Detailed Description Text (59):
Z, which may or may not be present, is a peptide or peptidomimetic having the formula (Xaa).sub.b, wherein Xaa is any amino acid or amino acid mimetic and b is an integer from 1 to 15; and

Detailed Description Text (62):
In this embodiment, the modulator compound is specifically modified at either its amino-terminus, its carboxy-terminus, or both. The terminology used in this formula is the same as described above. Suitable modifying groups are described in subsection II below. In one embodiment, the compound is modified only at its amino terminus (i.e., B is absent and the compound comprises the formula: A-(Y)-Xaa.sub.1,-Xaa.sub.2 -Xaa.sub.3 -Xaa.sub.4 -(Z)). In another embodiment, the compound is modified only at its carboxy-terminus (i.e., A is absent and the compound comprises the formula: (Y)-Xaa.sub.1 -Xaa.sub.2 -Xaa.sub.3 -Xaa.sub.4 -(Z)-B). In yet another embodiment, the compound is modified at both its amino- and carboxy termini (i.e., the compound comprises the formula: A-(Y)-Xaa.sub.1 -Xaa.sub.2 -Xaa.sub.3 -Xaa.sub.4 -(Z)-B and both A and B are present). As described above, the type and number of amino acid structures which flank the Xaa.sub.1 -Xaa.sub.2 -Xaa.sub.3 -Xaa.sub.4 core sequences in the above formula is variable. For example, in one embodiment, a and b are integers from 1 to 15. More preferably, a and b are integers between 1 and 10. Even more preferably, a and b are integers between 1 and 5. Most preferably, a and b are integers between 1 and 3.

Detailed Description Text (77):
In one specific embodiment, the compound comprises the formula: A-Xaa.sub.4 -Xaa.sub.5 -Xaa.sub.6 -Xaa.sub.7 -B (e.g, a modified form of A.beta..sub.17-20, comprising an amino acid sequence Leu-Val-Phe-Phe; SEQ ID NO:12).

Detailed Description Text (78):
In another specific embodiment, the compound comprises the formula: A-Xaa.sub.4 -Xaa.sub.5 -Xaa.sub.6 -Xaa.sub.7 -Xaa.sub.8 -B (e.g, a modified form of A.beta..sub.17-21, comprising an amino acid sequence Leu-Val-Phe-Phe-Ala; SEQ ID NO:11).

Detailed Description Text (79):
In another specific embodiment, the compound comprises the formula: A-Xaa.sub.3 -Xaa.sub.4 -Xaa.sub.5 -Xaa.sub.6 -Xaa.sub.7 -B (e.g., a modified form of A.beta..sub.16-20, comprising an amino acid sequence Lys-Leu-Val-Phe-Phe; SEQ ID NO:10).

Detailed Description Text (80):
In another specific embodiment, the compound comprises the formula: A-Xaa.sub.3 -Xaa.sub.4 -Xaa.sub.5 -Xaa.sub.6 -Xaa.sub.7 -Xaa.sub.8 -B (e.g., a modified form of A.beta..sub.16-21, comprising an amino acid sequence Lys-Leu-Val-Phe-Phe-Ala; SEQ ID NO:9).

Detailed Description Text (81):
In another specific embodiment, the compound comprises the formula: A-Xaa.sub.2 -Xaa.sub.3 -Xaa.sub.4 -Xaa.sub.5 -Xaa.sub.6 -Xaa.sub.7 -B (e.g., a modified form of

A.beta..sub.15-20, comprising an amino acid sequence Gln-Lys-Leu-Val-Phe-Phe; SEQ ID NO:8).

Detailed Description Text (82):
In another specific embodiment, the compound comprises the formula: A-Xaa.sub.2 -Xaa.sub.3 -Xaa.sub.4 -Xaa.sub.5 -Xaa.sub.6 -Xaa.sub.7 -Xaa.sub.8 -B (e.g., a modified form of A.beta..sub.15-21, comprising an acid sequence Gln-Lys-Leu-Val-Phe-Phe-Ala; SEQ ID NO:7).

Detailed Description Text (83):
In another specific embodiment, the compound comprises the formula: A-Xaa.sub.1 -Xaa.sub.2 -Xaa.sub.3 -Xaa.sub.4 -Xaa.sub.5 -Xaa.sub.6 -Xaa.sub.7 -B (e.g., a modified form of A.beta..sub.14-20, comprising an amino acid sequence His-Gln-Lys-Leu-Val-Phe-Phe; SEQ ID NO:6).

Detailed Description Text (84):
In another specific embodiment, the compound comprises the formula: A-Xaa.sub.1 -Xaa.sub.2 -Xaa.sub.3 -Xaa.sub.4 -Xaa.sub.5 -Xaa.sub.6 -Xaa.sub.7 -Xaa.sub.8 -B (e.g., a modified form of A.beta..sub.14-21, comprising an amino acid sequence His-Gln-Lys-Leu-Val-Phe-Phe-Ala; SEQ ID NO:5).

Detailed Description Text (86):
In further experiments to delineate subregions of A.beta. upon which an A.beta. aggregation core domain can be modeled (the results of which are described in Example 11), it was demonstrated that a modulator compound having inhibitory activity can comprise as few as three A.beta. amino acids residues (e.g., Val-Phe-Phe, which corresponds to A.beta..sub.8-20 or Phe-Phe-Ala, which corresponds to A.beta..sub.19-21). The results also demonstrated that a modulator compound having a modulating group at its carboxy-terminus is effective at inhibiting A.beta. aggregation. Still further, the results demonstrated that the cholyl group, as a modulating group, can be manipulated while maintaining the inhibitory activity of the compounds and that an iodotyrosyl can be substituted for phenylalanine (e.g., at position 19 or 20 of the A.beta. sequence) while maintaining the ability of the compound to inhibit A.beta. aggregation.

Detailed Description Text (87):
Still further, the results demonstrated that compounds with inhibitory activity can be created using amino acids residues that are derived from the A.beta. sequence in the region of about positions 17-21 but wherein the amino acid sequence is rearranged or has a substitution with a non-A.beta.-derived amino acid. Examples of such compounds include PPI-426, in which the sequence of A.beta..sub.17-21 (LVFFA SEQ ID NO:11) has been rearranged (FFVLA SEQ ID NO:21), PPI-372, in which the sequence of A.beta..sub.16-20 (KLVFF SEQ ID NO:10) has been rearranged (FKFVL SEQ ID NO:29), and PPI-388, -389 and -390, in which the sequence of A.beta..sub.17-21 (LVFFA SEQ ID NO:11) has been substituted at position 17, 18 or 19, respectively, with an alanine residue (AVFFA SEQ ID NO:25 for PPI-388, LAFFA SEQ ID NO:13 for PPI-389 and LVAFA SEQ ID NO:33 for PPI-390). The inhibitory activity of these compounds indicate that the presence in the compound of an amino acid sequence directly corresponding to a portion of A.beta. is not essential for inhibitory activity, but rather suggests that maintenance of the hydrophobic nature of this core region, by inclusion of amino acid residues such as phenylalanine, valine, leucine, regardless of their precise order, can be sufficient for inhibition of A.beta. aggregation. Accordingly, an A.beta. aggregation core domain can be designed based on the direct A.beta. amino acid sequence or can be designed based on a rearranged A.beta. sequence which maintains the hydrophobicity of the A.beta. subregion, e.g., the region around positions 17-20. This region of A.beta. contains the amino acid residues Leu, Val and Phe. Accordingly, preferred A.beta. aggregation core domains are composed of at least three amino acid structures (as that term is defined hereinbefore, including amino acid derivatives, analogues and mimetics), wherein at least two of the amino acid structures are, independently, either a leucine structure, a valine structure or a phenylalanine structure (as those terms are defined hereinbefore, including derivatives, analogues and mimetics).

Detailed Description Text (88):
Thus, in another embodiment, the invention provides a .beta.-amyloid modulator compound comprising a formula: ##STR9## wherein Xaa.sub.1, Xaa.sub.2 and Xaa.sub.3 are each amino acid structures and at least two of Xaa.sub.1, Xaa.sub.2 and Xaa.sub.3 are, independently, selected from the group consisting of a leucine structure, a phenylalanine structure and a valine structure;

Detailed Description Text (89):
Y, which may or may not be present, is a peptidic structure having the formula (Xaa).sub.a, wherein Xaa is any amino acid structure and a is an integer from 1 to 15;

Detailed Description Text (90):
Z, which may or may not be present, is a peptidic structure having the formula (Xaa).sub.b, wherein Xaa is any amino acid structure and b is an integer from 1 to 15; and

Detailed Description Text (95):
wherein Xaa.sub.1, Xaa.sub.2 and Xaa.sub.3 are each amino acid structures and at least two of Xaa.sub.1, Xaa.sub.2 and Xaa.sub.3 are, independently, selected from the group consisting of a leucine structure, a phenylalanine structure and a valine structure;

Detailed Description Text (96):
Y, which may or may not be present, is a peptidic structure having the formula (Xaa).sub.a, wherein Xaa is any amino acid structure and a is an integer from 1 to 15;

Detailed Description Text (97):
Z, which may or may not be present, is a peptidic structure having the formula (Xaa).sub.b, wherein Xaa is any amino acid structure and b is an integer from 1 to 15; and

Detailed Description Text (103):
In preferred specific embodiments, the invention provides a .beta.-amyloid modulator compound comprising a modifying group attached directly or indirectly to a peptidic structure, wherein the peptidic structure comprises amino acid structures having an amino acid sequence selected from the group consisting of His-Gln-Lys-Leu-Val-Phe-Phe-Ala (SEQ ID NO:5), His-Gln-Lys-Leu-Val-Phe-Phe (SEQ ID NO:6), Gln-Lys-Leu-Val-Phe-Phe-Ala (SEQ ID NO:7), Gln-Lys-Leu-Val-Phe-Phe (SEQ ID NO:8), Lys-Leu-Val-Phe-Phe-Ala (SEQ ID NO:9), Lys-Leu-Val-Phe-Phe (SEQ ID NO:10), Leu-Val-Phe-Phe-Ala (SEQ ID NO:11), Leu-Val-Phe-Phe (SEQ ID NO:12), Leu-Ala-Phe-Phe-Ala (SEQ ID NO:13), Val-Phe-Phe (SEQ ID NO:19), Phe-Phe-Ala (SEQ ID NO:20), Phe-Phe-Val-Leu-Ala (SEQ ID NO:21), Leu-Val-Phe-Phe-Lys (SEQ ID NO:22), Leu-Val-Iodotyrosine-Phe-Ala (SEQ ID NO:23), Val-Phe-Phe-Ala (SEQ ID NO:24), Ala-Val-Phe-Phe-Ala (SEQ ID NO:25), Leu-Val-Phe-Iodotyrosine-Ala (SEQ ID NO:26), Leu-Val-Phe-Phe-Ala-Glu (SEQ ID NO:27), Phe-Phe-Val-Leu (SEQ ID NO:28), Phe-Lys-Phe-Val-Leu (SEQ ID NO:29), Lys-Leu-Val-Ala-Phe (SEQ ID NO:30), Lys-Leu-Val-Phe-Phe-.beta.Ala (SEQ ID NO:31) and Leu-Val-Phe-Phe-DAla (SEQ ID NO:32).

Detailed Description Text (107):
Within a modulator compound of the invention, a peptidic structure (such as an A.beta. derived peptide, or an A.beta. aggregation core domain, or an amino acid sequence corresponding to a rearranged A.beta. aggregation core domain) is coupled directly or indirectly to at least one modifying group (abbreviated as MG). In one embodiment, a modulator compounds of the invention comprising an aggregation core domain coupled to a modifying group, the compound can be illustrated schematically as MG-ACD. The term "modifying group" is intended to include structures that are directly attached to the peptidic structure (e.g., by covalent coupling), as well as those that are indirectly attached to the peptidic structure (e.g., by a stable non-covalent association or by covalent coupling to additional amino acid residues, or mimetics, analogues or derivatives thereof, which may flank the A.beta.-derived peptidic structure). For example, the modifying group can be coupled to the amino-terminus or carboxy-terminus of an A.beta.-derived peptidic structure, or to a peptidic or peptidomimetic region flanking the core domain. Alternatively, the modifying group can be coupled to a side chain of at least one amino acid residue of an A.beta.-derived peptidic structure, or to a peptidic or peptidomimetic region flanking the core domain (e.g., through the epsilon amino group of a lysyl residue(s), through the carboxyl group of an aspartic acid residue(s) or a glutamic acid residue(s), through a hydroxy group of a tyrosyl residue(s), a serine residue(s) or a threonine residue(s) or other suitable reactive group on an amino acid side chain). Modifying groups covalently coupled to the peptidic structure can be attached by means and using methods well known in the art for linking chemical structures, including, for example, amide, alkylamino, carbamate or urea bonds.

Detailed Description Text (112):
A preferred polycyclic group is a group containing a cis-decalin structure. Although not intending to be limited by mechanism, it is thought that the "bent" conformation conferred on a modifying group by the presence of a cis-decalin structure contributes

to the efficacy of the modifying group in disrupting A.beta. polymerization. Accordingly, other structures which mimic the "bent" configuration of the cis-decalin structure can also be used as modifying groups. An example of a cis-decalin containing structure that can be used as a modifying group is a cholanoyl structure, such as a cholyl group. For example, a modulator compound can be modified at its amino terminus with a cholyl group by reacting the aggregation core domain with cholic acid, a bile acid, as described in Example 4 (the structure of cholic acid is illustrated in FIG. 2). Moreover, a modulator compound can be modified at its carboxy terminus with a cholyl group according to methods known in the art (see e.g., Wess, G. et al. (1993) Tetrahedron Letters, 34:817-822; Wess, G. et al. (1992) Tetrahedron Letters 33:195-198; and Kramer, W. et al. (1992) J. Biol. Chem. 267:18598-18604). Cholyl derivatives and analogues can also be used as modifying groups. For example, a preferred cholyl derivative is Aic (3-(O-aminoethyl-iso)-cholyl), which has a free amino group that can be used to further modify the modulator compound (e.g., a chelation group for .sup.99m Tc can be introduced through the free amino group of Aic). As used herein, the term "cholanoyl structure" is intended to include the cholyl group and derivatives and analogues thereof, in particular those which retain a four-ring cis-decalin configuration. Examples of cholanoyl structures include groups derived from other bile acids, such as deoxycholic acid, lithocholic acid, ursodeoxycholic acid, chenodeoxycholic acid and hyodeoxycholic acid, as well as other related structures such as cholanic acid, bufalin and resibufogenin (although the latter two compounds are not preferred for use as a modifying group). Another example of a cis-decalin containing compound is 5.beta.-cholestan-3.alpha.-ol (the cis-decalin isomer of (+)-dihydrocholesterol). For further description of bile acid and steroid structure and nomenclature, see Nes, W. R. and McKean, M. L. Biochemistry of Steroids and Other Isopentanoids, University Park Press, Baltimore, Md., Chapter 2.

Detailed Description Text (116):
Yet another type of modifying group is a compound that contains a non-natural amino acid that acts as a beta-turn mimetic, such as a dibenzofuran-based amino acid described in Tsang, K. Y. et al. (1994) J. Am. Chem. Soc. 116:3988-4005; Diaz, H. and Kelly, J. W. (1991) Tetrahedron Letters 41:5725-5728; and Diaz, H. et al. (1992) J. Am. Chem. Soc. 114:8316-8318. An example of such a modifying group is a peptide-aminoethyldibenzofuranyl-proprionic acid (Adp) group (e.g., DDIIL-Adp; SEQ ID NO:34). This type of modifying group further can comprise one or more N-methyl peptide bonds to introduce additional steric hindrance to the aggregation of natural .beta.-AP when compounds of this type interact with natural .beta.-AP.

Detailed Description Text (119):
To further chemically modify the compound, such as to alter the pharmacokinetic properties of the compound, reactive groups can be derivatized. For example, when the modifying group is attached to the amino-terminal end of the aggregation core domain, the carboxy-terminal end of the compound can be further modified. Preferred C-terminal modifications include those which reduce the ability of the compound to act as a substrate for carboxypeptidases. Examples of preferred C-terminal modifiers include an amide group, an ethylamide group and various non-natural amino acids, such as D-amino acids and .beta.-alanine. Alternatively, when the modifying group is attached to the carboxy-terminal end of the aggregation core domain, the amino-terminal end of the compound can be further modified, for example, to reduce the ability of the compound to act as a substrate for aminopeptidases.

Detailed Description Text (120):
A modulator compound can be further modified to label the compound by reacting the compound with a detectable substance. Suitable detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, .beta.-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; and examples of suitable radioactive material include .sup.14 C, .sup.123 I, .sup.124 I, .sup.125 I, .sup.131 I, .sup.99m Tc, .sup.35 S or .sup.3 H. In a preferred embodiment, a modulator compound is radioactively labeled with .sup.14 C, either by incorporation of .sup.14 C into the modifying group or one or more amino acid structures in the modulator compound. Labeled modulator compounds can be used to assess the in vivo pharmacokinetics of the compounds, as well as to detect A.beta. aggregation, for example for diagnostic purposes. A.beta. aggregation can be detected using a labeled modulator compound either in vivo or in an in vitro sample derived from a subject.

Detailed Description Text (121):
Preferably, for use as an in vivo diagnostic agent, a modulator compound of the invention is labeled with radioactive technetium or iodine. Accordingly, in one embodiment, the invention provides a modulator compound labeled with technetium, preferably .sup.99m Tc. Methods for labeling peptide compounds with technetium are known in the art (see e.g., U.S. Pat. Nos. 5,443,815, 5,225,180 and 5,405,597, all by Dean et al.; Stepniak-Biniakiewicz, D., et al. (1992) J. Med. Chem. 35:274-279; Fritzberg, A. R., et al. (1988) Proc. Natl. Acad. Sci. USA 85:4025-4029; Baidoo, K. E., et al. (1990) Cancer Res. Suppl. 50:799s-803s; and Regan, L. and Smith, C. K. (1995) Science 270:980-982). A modifying group can be chosen that provides a site at which a chelation group for .sup.99m Tc can be introduced, such as the Aic derivative of cholic acid, which has a free amino group (see Example 11). In another embodiment, the invention provides a modulator compound labeled with radioactive iodine. For example, a phenylalanine residue within the A.beta. sequence (such as Phe.sub.19 or Phe.sub.20) can be substituted with radioactive iodotyrosyl (see Example 11). Any of the various isotopes of radioactive iodine can be incorporated to create a diagnostic agent. Preferably, .sup.123 I (half-life=13.2 hours) is used for whole body scintigraphy, .sup.124 I (half life=4 days) is used for positron emission tomography (PET), .sup.125 I (half life=60 days) is used for metabolic turnover studies and 131I (half life=8 days) is used for whole body counting and delayed low resolution imaging studies.

Detailed Description Text (124):
Modulator compounds of the invention can be prepared by standard techniques known in the art. The peptide component of a modulator composed, at least in part, of a peptide, can be synthesized using standard techniques such as those described in Bodansky, M. Principles of Peptide Synthesis, Springer Verlag, Berlin (1993) and Grant, G. A. (ed.). Synthetic Peptides: A User's Guide, W. H. Freeman and Company, New York (1992). Automated peptide synthesizers are commercially available (e.g., Advanced ChemTech Model 396; Milligen/Biosearch 9600). Additionally, one or more modulating groups can be attached to the A.beta.-derived peptidic component (e.g., an A.beta. aggregation core domain) by standard methods, for example using methods for reaction through an amino group (e.g., the alpha-amino group at the amino-terminus of a peptide), a carboxyl group (e.g., at the carboxy terminus of a peptide), a hydroxyl group (e.g., on a tyrosine, serine or threonine residue) or other suitable reactive group on an amino acid side chain (see e.g., Greene, T. W. and Wuts, P. G. M. Protective Groups in Organic Synthesis, John Wiley and Sons, Inc., New York (1991)). Exemplary syntheses of preferred .beta. amyloid modulators is described further in Examples 1, 4 and 11.

Detailed Description Text (135):
Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, liposome, or other ordered structure suitable to high drug concentration. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, monostearate salts and gelatin. Moreover, the modulators can be administered in a time release formulation, for example in a composition which includes a slow release polymer. The active compounds can be prepared with carriers that will protect the compound against rapid release, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, polylactic acid and polylactic, polyglycolic copolymers (PLG). Many methods for the preparation of such formulations are patented or generally known to those skilled in the art.

Detailed Description Text (138):
In another embodiment, a pharmaceutical composition comprising a modulator of the invention is formulated such that the modulator is transported across the blood-brain barrier (BBB). Various strategies known in the art for increasing transport across the BBB can be adapted to the modulators of the invention to thereby enhance transport of the modulators across the BBB (for reviews of such strategies, see e.g., Pardridge, W. M. (1994) Trends in Biotechnol. 12:239-245; Van Bree, J. B. et al. (1993) Pharm. World

Sci. 15:2-9; and Pardridge, W. M. et al. (1992) Pharmacol. Toxicol. 71:3-10). In one approach, the modulator is chemically modified to form a prodrug with enhanced transmembrane transport. Suitable chemical modifications include covalent linking of a fatty acid to the modulator through an amide or ester linkage (see e.g., U.S. Pat. No. 4,933,324 and PCT Publication WO 89/07938, both by Shashoua; U.S. Pat. No. 5,284,876 by Hesse et al.; Toth, I. et al. (1 994) J. Drug Target. 2:217-239; and Shashoua, V. E. et al. (1984) J. Med. Chem. 27:659-664) and glycating the modulator (see e.g., U.S. Pat. No. 5,260,308 by Poduslo et al.). Also, N-acylamino acid derivatives may be used in a modulator to form a "lipidic" prodrug (see e.g., U.S. Pat. No. 5,112,863 by Hashimoto et al.).

Detailed Description Text (139):
In another approach for enhancing transport across the BBB, a peptidic or peptidomimetic modulator is conjugated to a second peptide or protein, thereby forming a chimeric protein, wherein the second peptide or protein undergoes absorptive-mediated or receptor-mediated transcytosis through the BBB. Accordingly, by coupling the modulator to this second peptide or protein, the chimeric protein is transported across the BBB. The second peptide or protein can be a ligand for a brain capillary endothelial cell receptor ligand. For example, a preferred ligand is a monoclonal antibody that specifically binds to the transferrin receptor on brain capillary endothelial cells (see e.g., U.S. Pat. Nos. 5,182,107 and 5,154,924 and PCT Publications WO 93/10819 and WO 95/02421, all by Friden et al.). Other suitable peptides or proteins that can mediate transport across the BBB include histones (see e.g., U.S. Pat. No. 4,902,505 by Pardridge and Schimmel) and ligands such as biotin, folate, niacin, pantothenic acid, riboflavin, thiamin, pryridoxal and ascorbic acid (see e.g., U.S. Pat. Nos. 5,416,016 and 5,108,921, both by Heinstein). Additionally, the glucose transporter GLUT-1 has been reported to transport glycopeptides (L-serinyl-.beta.-D-glucoside analogues of [Met5]enkephalin) across the BBB (Polt, R. et al. (1994) Proc. Natl. Acad. Sci. USA 91:7114-1778). Accordingly, a modulator compound can be coupled to such a glycopeptide to target the modulator to the GLUT-1 glucose transporter. For example, a modulator compound which is modified at its amino terminus with the modifying group Aic (3-(O-aminoethyl-iso)-cholyl, a derivative of cholic acid having a free amino group) can be coupled to a glycopeptide through the amino group of Aic by standard methods. Chimeric proteins can be formed by recombinant DNA methods (e.g., by formation of a chimeric gene encoding a fusion protein) or by chemical crosslinking of the modulator to the second peptide or protein to form a chimeric protein. Numerous chemical crosslinking agents are known in the (e.g., commercially available from Pierce, Rockford Ill.). A crosslinking agent can be chosen which allows for high yield coupling of the modulator to the second peptide or protein and for subsequent cleavage of the linker to release bioactive modulator. For example, a biotin-avidin-based linker system may be used.

Detailed Description Text (158):
In addition to the .beta.-amyloid modulators described hereinbefore in which an A.beta. peptide is coupled to a modifying group, the invention also provides .beta.-amyloid modulators comprised of an unmodified A.beta. peptide. It has now been discovered that certain portions of natural .beta.-AP can alter aggregation of natural .beta.-APs when contacted with the natural .beta.-APs (see Example 12). Accordingly, these unmodified A.beta. peptides comprise a portion of the natural .beta.-AP sequence (i.e., a portion of .beta.AP.sub.1-39, .beta.AP.sub.1-40, .beta.AP.sub.1-42 and .beta.AP.sub.1-43). In particular these unmodified A.beta. peptides have at least one amino acid deletion compared to .beta.AP.sub.1-39, the shortest natural .beta.-AP, such that the compound alters aggregation of natural .beta.-amyloid peptides when contacted with the natural .beta.-amyloid peptides. In various embodiments, these unmodified peptide compounds can promote aggregation of natural .beta.-amyloid peptides, or, more preferably, can inhibit aggregation of natural .beta.-amyloid peptides when contacted with the natural .beta.-amyloid peptides. Even more preferably, the unmodified peptide compound inhibits aggregation of natural .beta.-amyloid peptides when contacted with a molar excess amount of natural .beta.-amyloid peptides (e.g., a 10-fold, 33-fold or 100-fold molar excess amount of natural .beta.-AP).

Detailed Description Text (159):
As discussed above, the unmodified peptide compounds of the invention comprise an amino acid sequence having at least one amino acid deletion compared to the amino acid sequence of .beta.AP.sub.1-39. Alternatively, the unmodified peptide compound can have at least five, ten, fifteen, twenty, twenty-five, thirty or thirty-five amino acids deleted compared to .beta.AP.sub.1-39. Still further the unmodified peptide compound can have 1-5, 1-10, 1-15, 1-20, 1-25, 1-30 or 1-35 amino acids deleted compared to .beta.AP.sub.1-39. The amino acid deletion(s) may occur at the amino-terminus, the

carboxy-terminus, an internal site, or a combination thereof, of the .beta.-AP sequence. Accordingly, in one embodiment, an unmodified peptide compound of the invention comprises an amino acid sequence which has at least one internal amino acid deleted compared to .beta.AP.sub.1-39. Alternatively, the unmodified peptide compound can have at least five, ten, fifteen, twenty, twenty-five, thirty or thirty-five internal amino acids deleted compared to .beta.AP.sub.1-39. Still further the unmodified peptide compound can have 1-5, 1-10, 1-15, 1-20, 1-25, 1-30 or 1-35 internal amino acids deleted compared to .beta.AP.sub.1-39. For peptides with internal deletions, preferably the peptide has an amino terminus corresponding to amino acid residue 1 of natural .beta.AP and a carboxy terminus corresponding to residue 40 of natural .beta.AP and has one or more internal .beta.-AP amino acid residues deleted (i.e., a non-contiguous A.beta. peptide).

Detailed Description Text (160):
In another embodiment, the unmodified peptide compound comprises an amino acid sequence which has at least one N-terminal amino acid deleted compared to .beta.AP.sub.1-39. Alternatively, the unmodified peptide compound can have at least five, ten, fifteen, twenty, twenty-five, thirty or thirty-five N-terminal amino acids deleted compared to .beta.AP.sub.1-39. Still further the unmodified peptide compound can have 1-5, 1-10, 1-15, 1-20, 1-25, 1-30 or 1-35 N-terminal amino acids deleted compared to .beta.AP.sub.1-39.

Detailed Description Text (161):
In yet another embodiment, the unmodified peptide compound comprises an amino acid sequence which has at least one C-terminal amino acid deleted compared to .beta.AP.sub.1-39 Alternatively, the unmodified peptide compound can have at least five, ten, fifteen, twenty, twenty-five, thirty or thirty-five C-terminal amino acids deleted compared to .beta.AP.sub.1-39. Still further the unmodified peptide compound can have 1-5, 1-10, 1-15, 1-20, 1-25, 1-30 or 1-35 C-terminal amino acids deleted compared to .beta.AP.sub.1-39.

Detailed Description Text (162):
In addition to deletion of amino acids as compared to .beta.AP.sub.1-39, the peptide compound can have additional non-.beta.-AP amino acid residues added to it, for example, at the amino terminus, the carboxy-terminus or at an internal site. In one embodiment, the peptide compound has at least one non-.beta.-amyloid peptide-derived amino acid at its N-terminus. Alternatively, the compound can have, for example, 1-3, 1-5, 1-7, 1-10, 1-15 or 1-20 non-.beta.-amyloid peptide-derived amino acid at its N-terminus. In another embodiment, the peptide compound has at least one non-.beta.-amyloid peptide-derived amino acid at its C-terminus. Alternatively, the compound can have, for example, 1-3, 1-5, 1-7, 1-10, 1-15 or 1-20 non-.beta.-amyloid peptide-derived amino acid at its C-terminus.

Detailed Description Text (163):
In specific preferred embodiments, an unmodified peptide compound of the invention comprises A.beta..sub.6-20 (the amino acid sequence of which is shown in SEQ ID NO:4), A.beta..sub.16-30 (the amino acid sequence of which is shown in SEQ ID NO:14), A.beta..sub.1-20, 26-40 (the amino acid sequence of which is shown in SEQ ID NO:15) or EEVVHHHHQQ-.beta.AP.sub.16-40 (the amino acid sequence of which is shown in SEQ ID NO:16). In the nomenclature used herein, .beta.AP.sub.1-20, 26-40 represents .beta.AP.sub.1-40 in which the internal amino acid residues 21-25 have been deleted.

Detailed Description Text (164):
An unmodified peptide compound of the invention can be chemically synthesized using standard techniques such as those described in Bodansky, M. Principles of Peptide Synthesis, Springer Verlag, Berlin (1993) and Grant, G. A. (ed.). Synthetic Peptides: A User's Guide, W. H. Freeman and Company, New York (1992). Automated peptide synthesizers are commercially available (e.g., Advanced ChemTech Model 396; Milligen/Biosearch 9600). Alternatively, unmodified peptide compounds can be prepared according to standard recombinant DNA techniques using a nucleic acid molecule encoding the peptide. A nucleotide sequence encoding the peptide can be determined using the genetic code and an oligonucleotide molecule having this nucleotide sequence can be synthesized by standard DNA synthesis methods (e.g., using an automated DNA synthesizer). Alternatively, a DNA molecule encoding an unmodified peptide compound can be derived from the natural .beta.-amyloid precursor protein gene or cDNA (e.g., using the polymerase chain reaction and/or restriction enzyme digestion) according to standard molecular biology techniques.

Detailed Description Text (165):

Accordingly, the invention further provides an isolated nucleic acid molecule comprising a nucleotide sequence encoding a .beta.-amyloid peptide compound, the .beta.-amyloid peptide compound comprising an amino acid sequence having at least one amino acid deletion compared to .beta.AP.sub.1-39 such that the .beta.-amyloid peptide compound alters aggregation of natural .beta.-amyloid peptides when contacted with the natural .beta.-amyloid peptides. As used herein, the term "nucleic acid molecule" is intended to include DNA molecules and RNA molecules and may be single-stranded or double-stranded, but preferably is double-stranded DNA. The isolated nucleic acid encodes a peptide wherein one or more amino acids are deleted from the N-terminus, C-terminus and/or an internal site of .beta.AP.sub.1-39, as discussed above. In yet other embodiments, the isolated nucleic acid encodes a peptide compound having one or more amino acids deleted compared to .beta.AP.sub.1-39 and further having at least one non-.beta.-AP derived amino acid residue added to it, for example, at the amino terminus, the carboxy-terminus or at an internal site. In specific preferred embodiments, an isolated nucleic acid molecule of the invention encodes .beta.AP.sub.6-20, .beta.AP.sub.16-30, .beta.AP.sub.1-20, 26-40 or EEVVHHHHQQ-.beta.AP.sub.16-40 (SEQ ID NO:16).

Detailed Description Text (166):
To facilitate expression of a peptide compound in a host cell by standard recombinant DNA techniques, the isolated nucleic acid encoding the peptide is incorporated into a recombinant expression vector. Accordingly, the invention also provides recombinant expression vectors comprising the nucleic acid molecules of the invention. As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant expression vectors" or simply "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" may be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors, which serve equivalent functions.

Detailed Description Text (167):
In the recombinant expression vectors of the invention, the nucleotide sequence encoding the peptide compound are operatively linked to one or more regulatory sequences, selected on the basis of the host cells to be used for expression. The term "operably linked" is intended to mean that the sequences encoding the peptide compound are linked to the regulatory sequence(s) in a manner that allows for expression of the peptide compound. The term "regulatory sequence" is intended to includes promoters, enhancers and other expression control elements (e.g., polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel; Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, Calif. (1990). Regulatory sequences include those that direct constitutive expression of a nucleotide sequence in many types of host cell, those that direct expression of the nucleotide sequence only in certain host cells (e.g., tissue-specific regulatory sequences) and those that direct expression in a regulatable manner (e.g., only in the presence of an inducing agent). It will be appreciated by those skilled in the art that the design of the expression vector may depend on such factors as the choice of the host cell to be transformed, the level of expression of peptide compound desired, etc. The expression vectors of the invention can be introduced into host cells thereby to produce peptide compounds encoded by nucleic acids as described herein.

Detailed Description Text (170):
A recombinant expression vector comprising a nucleic acid encoding a peptide compound that alters aggregation of natural .beta.-AP can be introduced into a host cell to thereby produce the peptide compound in the host cell. Accordingly, the invention also provides host cells containing the recombinant expression vectors of the invention. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in

succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein. A host cell may be any prokaryotic or eukaryotic cell. For example, a peptide compound may be expressed in bacterial cells such as E. coli, insect cells, yeast or mammalian cells. Preferably, the peptide compound is expressed in mammalian cells. In a preferred embodiment, the peptide compound is expressed in mammalian cells in vivo in a mammalian subject to treat amyloidosis in the subject through gene therapy (discussed further below). Preferably, the .beta.-amyloid peptide compound encoded by the recombinant expression vector is secreted from the host cell upon being expressed in the host cell.

Detailed Description Text (171):
Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (e.g., DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, electroporation, microinjection and viral-mediated transfection. Suitable methods for transforming or transfecting host cells can be found in Sambrook et al. (Molecular Cloning: A Laboratory Manual, 2nd Edition, Cold Spring Harbor Laboratory press (1989)), and other laboratory manuals. Methods for introducing DNA into mammalian cells in vivo are also known in the art and can be used to deliver the vector DNA to a subject for gene therapy purposes (discussed further below).

Detailed Description Text (172):
For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (e.g., resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Preferred selectable markers include those that confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid encoding a selectable marker may be introduced into a host cell on the same vector as that encoding the peptide compound or may be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (e.g., cells that have incorporated the selectable marker gene will survive, while the other cells die).

Detailed Description Text (173):
A nucleic acid of the invention can be delivered to cells in vivo using methods known in the art, such as direct injection of DNA, receptor-mediated DNA uptake or viral-mediated transfection. Direct injection has been used to introduce naked DNA into cells in vivo (see e.g., Acsadi et al. (1991) Nature 332:815-818; Wolff et al. (1990) Science 247:1465-1468). A delivery apparatus (e.g., a "gene gun") for injecting DNA into cells in vivo can be used. Such an apparatus is commercially available (e.g., from BioRad). Naked DNA can also be introduced into cells by complexing the DNA to a cation, such as polylysine, which is coupled to a ligand for a cell-surface receptor (see for example Wu, G. and Wu, C. H. (1988) J. Biol. Chem. 263:14621; Wilson el al. (1992) J. Biol. Chem. 267:963-967; and U.S. Pat. No. 5,166,320). Binding of the DNA-ligand complex to the receptor facilitates uptake of the DNA by receptor-mediated endocytosis. Additionally, a DNA-ligand complex linked to adenovirus capsids which naturally disrupt endosomes, thereby releasing material into the cytoplasm can be used to avoid degradation of the complex by intracellular lysosomes (see for example Curiel el al. (1991) Proc. Natl. Acad. Sci. USA 88:8850; Cristiano et al. (1993) Proc. Natl. Acad. Sci. USA 90:2122-2126).

Detailed Description Text (176):
Adeno-associated virus (AAV) can also be used for delivery of DNA for gene therapy purposes. AAV is a naturally occurring defective virus that requires another virus, such as an adenovirus or a herpes virus, as a helper virus for efficient replication and a productive life cycle. (For a review see Muzyczka et al. Curr. Topics in Micro. and Immunol. (1992) 158:97-129). It is also one of the few viruses that may integrate its DNA into non-dividing cells, and exhibits a high frequency of stable integration (see for example Flotte et al. (1992) Am. J. Respir. Cell. Mol. Biol. 7:349-356; Samulski et al. (1989) J. Virol. 63:3822-3828; and McLaughlin et al. (1989) J. Virol. 62:1963-1973). Vectors containing as little as 300 base pairs of AAV can be packaged and can integrate. An AAV vector such as that described in Tratschin et al. (1985) Mol. Cell. Biol. 5:3251-3260 can be used to introduce DNA into cells. A variety of nucleic acids have been introduced into different cell types using AAV vectors (see for example Hermonat et al. (1984) Proc. Natl. Acad. Sci. USA 81:6466-6470; Tratschin et al. (1985)